Figure S1. Construction of a lentiviral vector for delivering shRNA against human CCR5

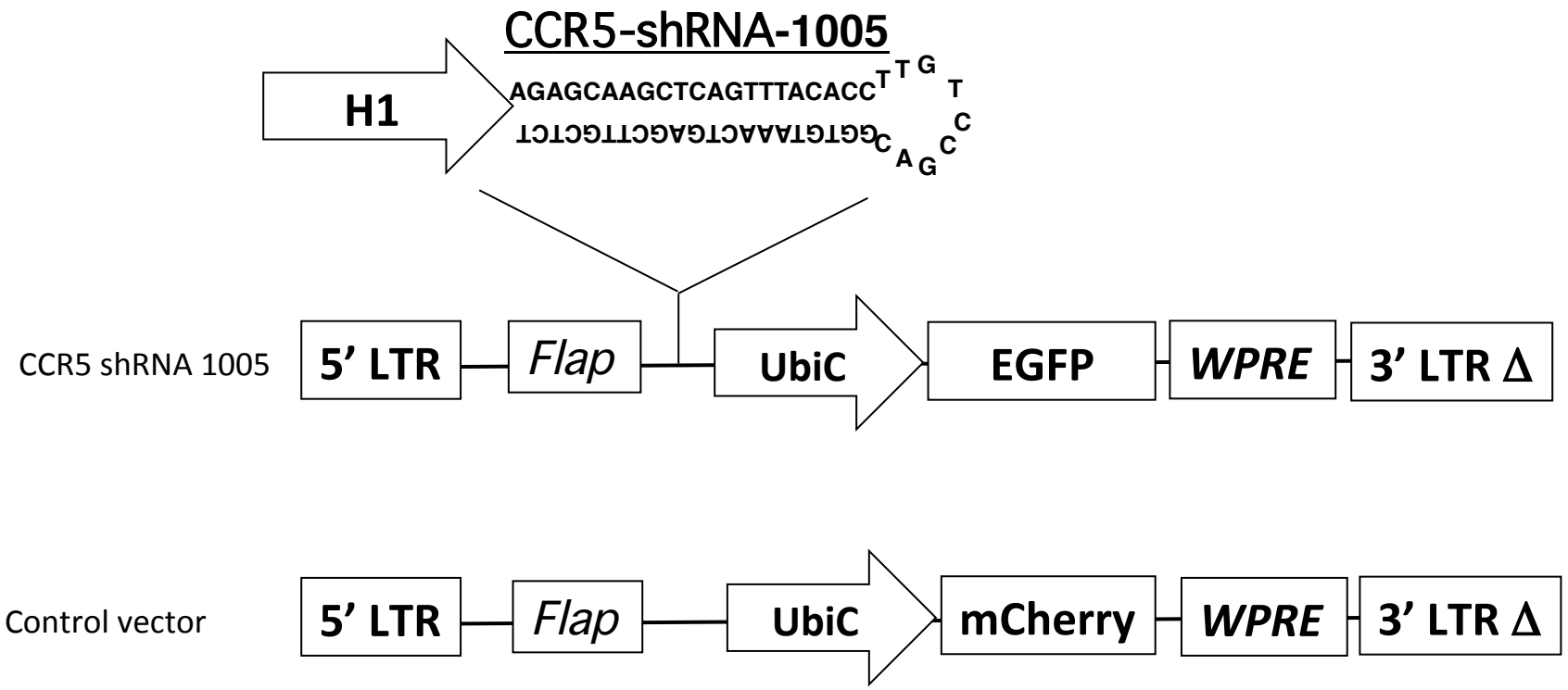

Figure S1. Construction of a lentiviral vector for delivering shRNA against human CCR5.
Schematic diagram of the shRNA-expressing lentiviral vector. A short hairpin RNA (1005) against human CCR5 is expressed under the control of a human H1 RNA Pol III promoter (H1). The vector also contains a human Ubiquitin C (UbiC) promoter driving the EGFP or mCherry marker gene expression for tracking transduced cells. 5' LTR: HIV-1 5' long terminal repeat: 3' LTRD: HIV-1 self-inactivating 3' LTR; Flap: HIV-1 DNA flap element; WPRE: woodchuck hepatitis B virus RNA regulatory element.

Figure S2. Mouse experimental design

Figure S2. Mouse experimental design.
(a)Donor 1 was used for Experiment 1 (Ex1), Donor 2,3,and 4 were used for Experiment 2 (Ex2) and Donor 5 was used for Experiment 3 (Ex3).
(b)Transduction efficiency was measured by FC500 (Ex1) , LSRII (Ex2) and Fortessa (Ex3) after 5 days culture with IL-3, IL-6 and stem cell factor.

(a)

	Myeloablation procedure	MOI	Transduction (EGFP)	Transduction (mCherry)	Mice used for transplant	Mice died before HIV challenge	Mice used for HIV challenged
Ex1	300cGy TBI	2	26.2%	9.2 %	10	1	9
Ex2	35mg/kg Busulfan	3	71.3% 84.5% 81.4%	55.8% 62.5% 71.8%	15	2	13
Ex3	25mg/kg x 2times Busulfan	4	53.4%	62.3%	15	1	14

(b)

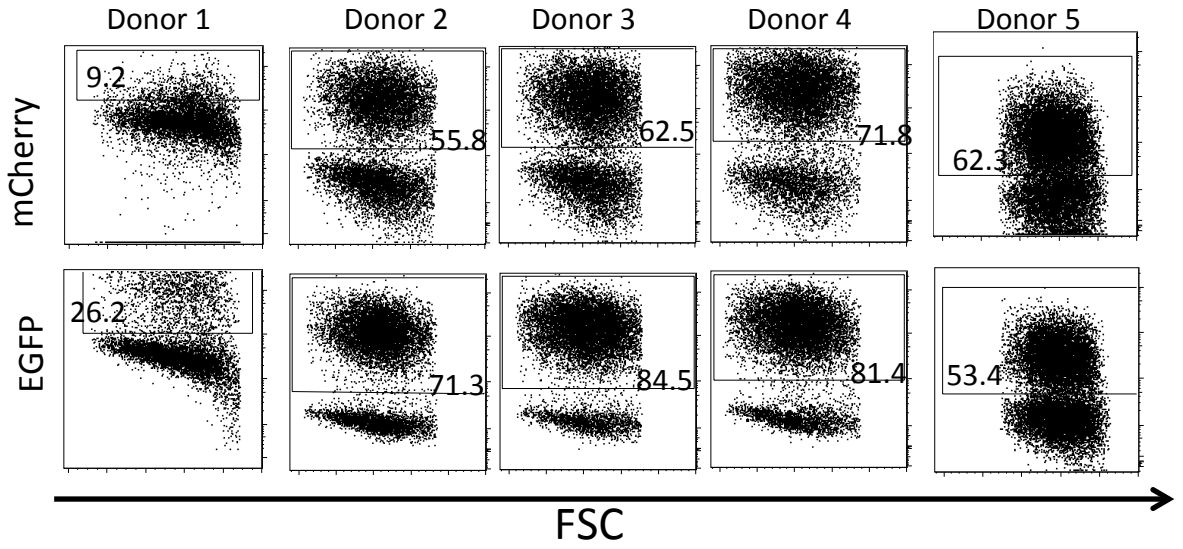

Figure S3. The percentage of human CD45 in Lymphocytes population at week 8.

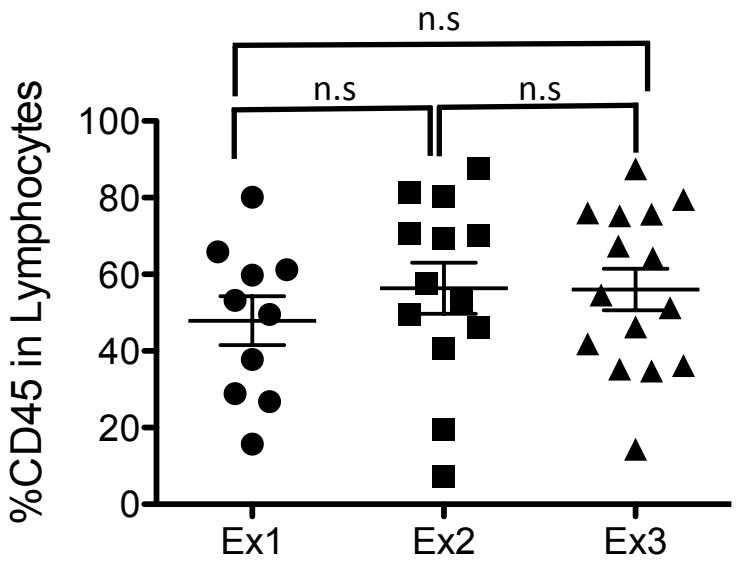

Figure S3. The percentage of human CD45 in Lymphocytes population at week 8.
Mouse whole blood was stained with anti-human CD45 antibody to compare human reconstitution levels in the three different groups at 8 weeks post human ThyLiv implantation. There were no significant differences in human reconstitution between the three groups.

Figure S4. CD4/CD8 ratio in sh1005 modified naïve T cells in lymphoid organs

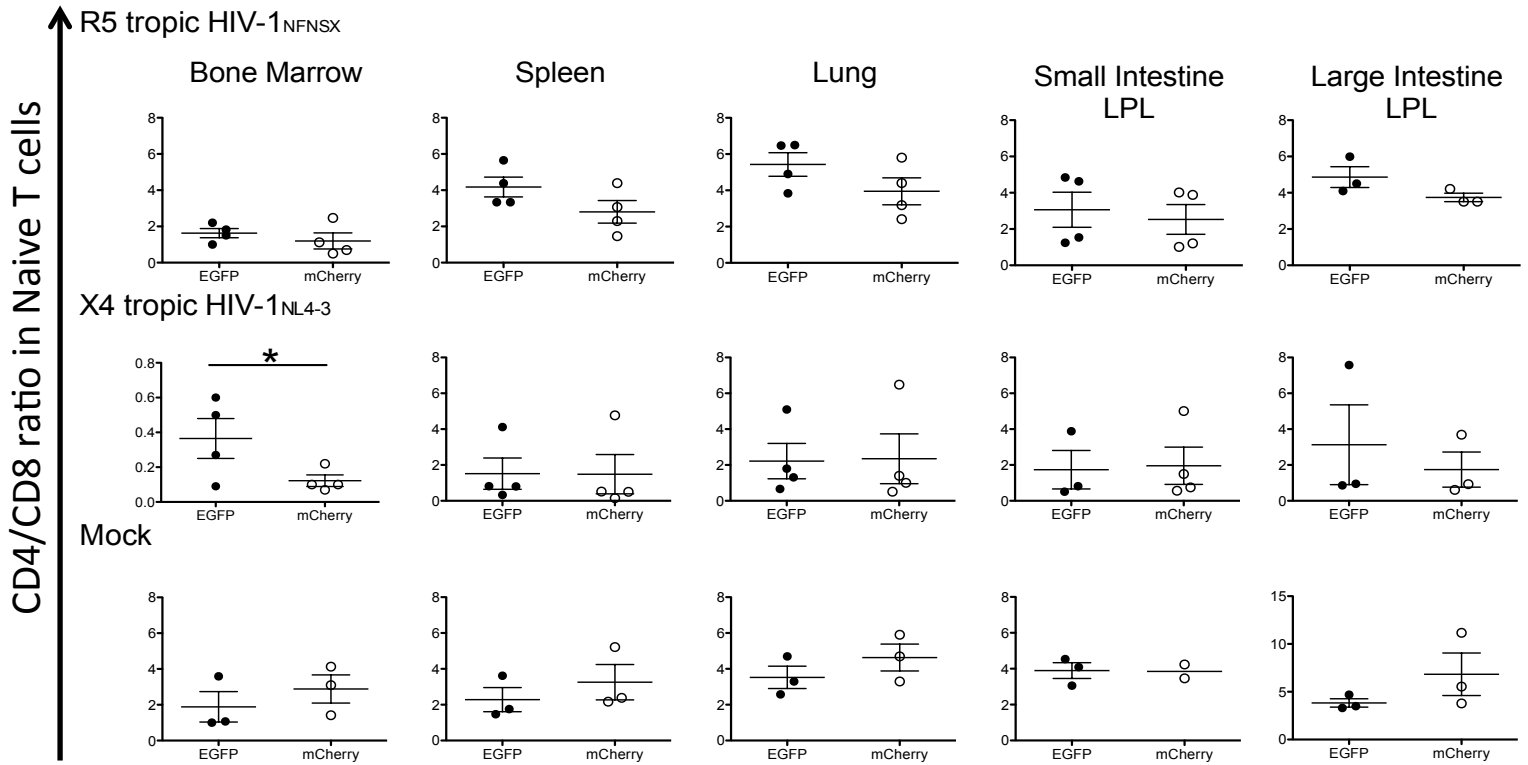

Figure S4. CD4/CD8 ratio in sh1005 modified naïve T cells in lymphoid organs

Comparison of the level of % naïve T lymphocytes in EGFP+ (solid circle) and mCherry+ (open circle) from all analyzed mice (HIV-1_{NFNSX} infected mice; n=4, HIV-1_{NL4-3} infected mice; n=4, Mock infected mice; n=3). A bar represents the mean value.

Figure S5. Comparison of CCR5 expression levels between EGFP+ and mCherry+ memory CD4T+ cells

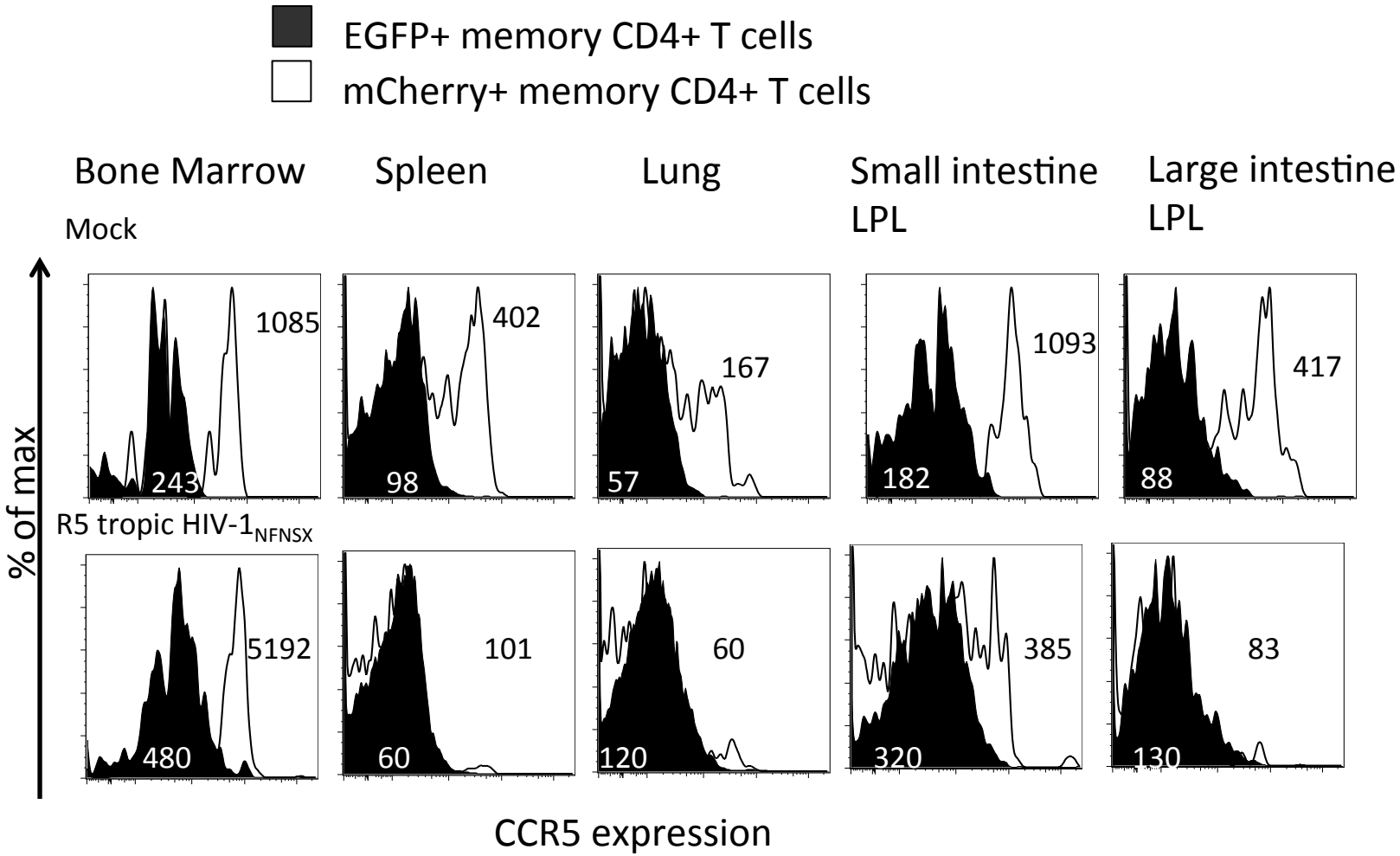

Figure S5. Comparison of CCR5 expression levels between EGFP+ and mCherry+ memory CD4T+ cells.
FACS histogram plots showing comparison of CCR5 expression in mock and an R5 tropic HIV-1 challenged mouse. Open curve indicates CCR5 expression in mCherry+ memory CD4+ T cells and solid curve indicates CCR5 expression in EGFP+ memory CD4+ T cells from an R5 tropic HIV-1 challenged mouse. Numbers refer to mean fluorescent intensity (MFI).

Table S1. No co-receptor changing.

		% of EGFP (Average ±SD)
	Mock	0.262±0.2
X4 tropic HIV-1	HIV-1 $_{NL4-3}$	29.4±1.8
R5 tropic HIV-1	HIV-1 $_{NFNSX}$	0.245±0.16
X4 tropic HIV-1 infected	mouse 1	18.7±1.1
	mouse 2	16.2±1.0
	mouse 3	6.29±0.13
R5 tropic HIV-1 infected	mouse 4	0.214±0.05
	mouse 5	0.302±0.16
	mouse 6	0.311±0.25

Table S1.measureing co-receptor changing
Mouse splenocytes were cultured with human PBMC with stimulation. Three days after incubation, cell culture supernatant were collected and cultured with Ghost(3)CXCR4 or Ghost(3) Hi-5 cells. After two days, the percentage of EGFP in cells were measured and analyzed with flow cytometry.